paper. For the present it seems best to regard this organ as simply a sporangium-pedicel, though there is no analogy among known Cryptogams for the presence of a vascular bundle in the stalk of a sporangium.

It appears that all species of *Sphenophyllum* in which the fructification is known, had essentially similar strobili, with pedicellate sporangia.

The genus *Sphenophyllum* cannot be placed in any existing family of Vascular Cryptogams. Anatomically there are some striking points of resemblance to Lycopodiaceæ, but the habit and fructification are totally different from anything in that order. *Sphenophyllum*, in fact, constitutes a group by itself, which is entirely unrepresented at the present epoch, and the affinities of which cannot be determined until additional forms have been discovered.

The paper is illustrated by numerous photographs from the actual preparations and specimens, and by a long series of camera-lucida drawings, executed by Mr. George Brebner.

II. "Researches on the Germination of the Pollen Grain and the Nutrition of the Pollen Tube." By J. REYNOLDS GREEN, M.A., B.Sc., Professor of Botany to the Pharmaceutical Society of Great Britain. Communicated by W. T. THISELTON DYER, F.R.S., C.M.G., C.I.E. Received January 2, 1894.

(From the Jodrell Laboratory, Royal Gardens, Kew.)

(Abstract.)

Many observers, especially Van Tieghem and Mangin, have established the fact that the growth of the pollen tube is a process of true germination, strictly comparable to that of the growth of the prothallus from the spore in the groups of Vascular Cryptogams. The germinative process is carried on at the expense of various reserve materials deposited partly in the pollen grain itself and partly in the conducting tissue of the style, down which the pollen tube makes its way.

The existence of certain enzymes in the pollen grain has also been proved by Van Tieghem and by Strasburger. The former has shown that when the pollen of several genera, especially *Crocus* and *Narcissus*, is cultivated in cane-sugar solutions, a certain amount of grape-sugar is produced in the culture, suggesting the presence of invertase; while the latter has shown similarly that certain pollens, when cultivated in starch paste, can liquefy it, with the formation of maltose.

The object of the present research was to isolate these enzymes and to investigate any changes in the amount of either of them during the progress of the germination. Further, to ascertain something of the metabolism going on in both the pollen grain and the style in the interval between pollination and fertilisation.

Both the enzymes were prepared from bruised grains by the use of the ordinary solvents—water, glycerine, and solutions of common salt—the latter being, perhaps, the most efficient. Details of the experiments, and the conditions of extraction, are given in the paper of which this is an abstract. Diastase was found in the resting pollen of various species of *Lilium*, *Helianthus*, *Gladiolus*, *Anemone*, *Antirrhinum*, *Tropœolum*, *Pelargonium*, *Crocus*, *Brownea*, *Helleborus*, *Alnus*, *Tulipa*, and *Clivia*, and in that of *Zamia* after germination had begun. The diastase is in the form of the translocation diastase of Brown and Morris. Invertase was found in the pollen of *Helleborus*, *Narcissus*, *Richardia*, *Lilium*, and *Zamia*. Some of these species contained both enzymes.

During the germination of the pollen grain the quantity of both enzymes was found to show a considerable increase in amount, in some cases even four or five fold. This increase was estimated by noting the diastatic or invertive power of extracts prepared side by side, from weighed quantities of pollen, and from equal quantities allowed to germinate in various culture fluids. Control experiments were carried out to show that the increase of enzyme action was not due to a more complete extraction of the ferment from the thin-walled tube than was possible from the thick-walled grain, but that there was an actual total increase of the enzyme. In one case there was noticed a diminution of ferment in the earliest stages of the germination, which may probably be correlated with the digestion of the starch grains of the grain, or of some portion of them, before the output of the pollen tube. It was found, further, that when the power of germination of the pollen grain was becoming feeble, from its being kept for some weeks, there was a very considerable diminution of the amount of diastase that could be extracted. Full details of these experiments are given in the paper.

The mode of growth and nutrition of the pollen tube was investigated by culture of the grain in hanging drops of fluid in a moist chamber, and by chemical analysis of the contents of various pollen grains and styles.

The microscopic examination of pollen tubes revealed general granularity of their contents, with the formation of certain large and refringent granules, that were apparently extruded regularly by the tube at definite places near the top. This appearance has already been noticed by Van Tieghem, to whose work reference is made. The extrusion of these granules suggests that they are the medium

of excretion of the enzyme, which can readily be detected in the culture fluid.

The reserve stores of the pollen grain differ in various species. They include starch, possibly in some cases dextrin, cane sugar, maltose, and glucose. The intracellular action of diastase can be noticed in the cases in which it is present, by the transformation of the starch granules as they pass along the tube, iodine staining them blue in the grain and upper part of the tube, then purple, and finally almost red as the tip is approached; indicating thus the gradually increasing formation of dextrin, one of the accompaniments of starch digestion. Quantitative estimations of the sugars are quoted in detail in the paper.

The distribution of starch in the style of the Lily was found to have a close relation to the progress of the pollen tube. The cells lining the cavity of the style, and the cells of the loose conducting tissue abutting on it, were found to contain starch grains, in greater or less amounts, varying with the species and with the age of the individual style. The outer soft tissue of the fibro-vascular bundles of this organ were also charged with crowds of starch grains, indicating a transport of this reserve material from the leaves. Cane sugar, maltose, and possibly glucose, were found to be the sugars present in the various styles examined.

In longitudinal preparations the starch was found to stop short some few mm. below the stigma, suggesting the view that the reserves in the style are intended to supplement those in the pollen grain, the latter being utilised in the early stages of germination.

Not only reserve materials can be found in the style, but in certain cases diastase also exists.

The action of the enzymes of the pollen is thus found to be partly intracellular, digesting the contents of the pollen grain, and partly extracellular, being excreted into the tissue of the style to work upon the external reserves. This is particularly noteworthy in the case of *Narcissus*, where the grain contains invertase, but, according to Van Tieghem, no cane sugar. The latter is found in considerable quantity in the style.

The development of the enzyme is not a phenomenon of starvation. The increased production noted is partly an effect of the absorption of food material which appears to act as a stimulant to its production.

Evidence on this point is quoted in the paper.

The absorption of food material often leads to an increase of starch in the grain and in the tube.

The increase of enzyme noted in certain cases lends a certain amount of support to the view that the enzyme exists in the pollen grain in the form of a zymogen. Some evidence bearing out this

view is derived from some experiments on the pollen of *Zamia*. A watery extract of this pollen was found to have no diastatic power, but on being warmed with a little malic acid for some hours, and then neutralised, it was found to have acquired a feeble one, very slowly hydrolysing some thin starch paste. Further experiments upon this point are, however, necessary before pronouncing decidedly that the zymogen exists.

The whole of the researches may be summarised as under:—

1. Diastase and invertase are both present in pollen grains, and can be extracted from them by the same treatment as has been found effectual in the cases of seeds and foliage leaves. The relative quantities vary a good deal; while some pollens contain both, others possess only one, which may be either of the two.
2. At the onset of germination the amount of both diastase and invertase is usually considerably increased. In one species examined this increase was preceded by a primary diminution. When the pollen grain has lost the power of germinating, the quantity of diastase has considerably decreased.
3. The pollen tube is nourished during its growth by plastic reserve material derived from two sources, the store of material in the grain itself, and a further store deposited in the style.
4. The reserve store of the pollen grain consists of different materials in different species: starch, dextrin, cane sugar, maltose, and glucose being the forms in which it is found.
5. The store in the style consists usually of the same carbohydrates, with the exception of dextrin.
6. The style itself contains enzymes to assist in preparing the reserve materials for absorption by the pollen tube, while the latter excretes the same ferments during its progress down the conducting tissue.
7. The absorption of food material appears to be one cause of the increase of enzyme found to occur during the germination.
8. This absorption of food material is usually so active that the reserve store of the pollen grain is often largely increased by a temporary deposition, either in the grain or its tube, of some of the absorbed sugar in the form of starch.
9. There is a certain amount of evidence pointing to the existence of zymogens in some pollens, particularly such as germinate in a faintly acid medium.